Lipid Oxidation in Heat-Sterilized Beef [a]

SUMMARY

Lipid oxidation decreases as the internal temperature of ground beef round is increased. The production of antioxidant-active substances is responsible for the oxidative stability. The quantity of total lipids or polyunsaturated fatty acids was not markedly changed by extensive heat treatment. Pigment destruction was progressive as heat treatment increased, but in no case was the pigment destroyed completely. Dilute slurries of overcooked beef have a pleasant "meaty" odor and may have some use as cover solutions for cooked meats.

LIPID OXIDATION in uncured meats develops rapidly after heating (Tims and Watts, 1958), and the acceptability of the meat decreases markedly in hours (Chang *et al.*, 1961). Studies of this nature have been carried out on meats cooked to internal temperatures of 70–80°C. However, preliminary observations have indicated that the rate of lipid oxidation was much lower in heat-sterilized beef than in beef heated only to the above temperatures (Florida State Univ., unpublished). Since many canned (heat-sterilized) meat products, without added antioxidants, are on the market, it is of interest to make a systematic investigation of the oxidative reaction in meat heated to progressively higher internal temperatures.

It has been established that rancidity results from the oxidative decomposition of unsaturated fatty acids. The lipid fractions involved primarily are the proteolipids and phospholipids rather than the triglycerides (Younathan and Watts, 1960). Younathan and Watts (1959) recently postulated that the ferric hemichromogen formed in fresh meat under the influence of heat, acts as an active catalyst for fat oxidation.

Several hypotheses were considered as possible explanations for less oxidation in overheated meats. Destruction of heme pigments, resulting in loss of the active catalyst, in the heat-sterilization process might contribute to the slower oxidation in such products. Alternatively, interaction of the reactive lipids with heme pigments or the oxidation of these lipids with subsequent disappearance of the oxidation products, resulting in a loss of oxidizable substrate, might take place. A significant and progressive loss of extractable lipids has been observed in this laboratory in the lateral line tissue of mullet as oxidation proceeds. A third possibility was the production of an antioxidant strong enough to prevent oxidation, in the tissue itself during the heat treatment.

The work reported was designed to investigate the effect of higher heat treatments on lipid oxidation in beef. Pigment destruction, lipid destruction, and antioxidant production were all investigated to some degree to determine the reason for the oxidative stability of extensively heated samples.

METHODS

Preparation of meat. Fresh beef round was used exclusively. Round steak was trimmed of excess fat and ground in Ward's Electric Food Chopper, Model VGS-5169A. Portions of 175 g each were weighed into 307 x 113 C-enameled cans, which were sealed immediately. The cans of meat were heated in an autoclave, which was opened after various periods. The temperature indicated on a thermometer in a control can was recorded as the internal temperature of the samples being removed at that time. The cans were cooled in a running water bath. They were then opened and the contents mixed and placed in bowls, which were covered with aluminum foil and refrigerated (5–7°C).

Tissue lipid oxidation. The 2-thiobarbituric acid test (Tarladgis *et al.*, 1960) was used to measure tissue lipid oxidation during various storage periods. Duplicate determinations were made, and the average was reported as the "TBA number," i.e., mg of malonaldehyde per 1000 g meat.

Concentration of myoglobin. Total pigments were determined by the acetone extraction procedure of Hornsey (1956) as modified by Gantner (1960). Duplicate determinations were made, and the average was reported as g/100 g meat.

Lipid content. The polyunsaturated fatty acid content of beef samples was determined by extracting total lipids (Folch *et al.*, 1957) and assaying for polyunsaturated fatty acids according to the lipoxidase method of MacGee (1959). The concentration of polyunsaturated fatty acids is reported in moles rather than as percent. The percent calculation proposed by MacGee makes assumptions of the mean molecular weight of the fatty acids that would probably not be valid for muscle lipids.

Organoleptic evaluation. The intensity of rancid odor was rated by a panel of trained judges, and the results were evaluated statistically as described by Tarladgis *et al.* (1959). Differences that were significant or highly significant are indicated in the tables. The judges were encouraged to comment on odors other than rancidity that may have been present.

EXPERIMENTAL RESULTS

Lipid oxidation and heme destruction at higher temperature. This experiment was designed to determine the extent of lipid oxidation and the extent of myoglobin destruction in ground beef round samples heated to progressively higher internal temperatures. The temperatures, 80, 95, 99, and 110°, were achieved by putting all samples in the autoclave at the same time, and removing the cans individually after various periods. An "overcooked" sample was secured by leaving meat in the autoclave for 1 hr after the 110°C sample had been removed. The meat was removed from the cans just after cooling, and samples for the TBA test and pigment extraction were taken initially and after 3 and 8 days of refrigerated storage. The data are in Table 1.

[a] A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service. Also supported in part by a research grant, RG-6885, from the National Institutes of Health, U. S. Public Health Service.

Table 1. TBA numbers and myoglobin retention of beef ound heated to several internal temperatures.

Internal emperature	Days of storage	TBA number	Myoglobin retention [a] %	Sensory score [b]
80°C	0	1.0	100	....
	3	11.	81	2.9
	8	19.	51	2.6(a)
95°C	0	0.9	97	....
	3	7.6	69	....
	8	15.	41	....
99°C	0	0.8	83	....
	3	5.2	70	....
	8	12.	45	....
10°C	0	0.5	76	....
	3	0.7	64	5.8
	8	1.2	61	5.1(b)
10°C + 1 hr at 110°C	0	0.2	34	....
	3	0.2	30	5.3
	8	0.3	32	5.9(c)

[a] Based on the myoglobin value of 0.44 g/100 g meat for 80°C at 0 lays storage.

[b] Based on a scale of 1–6 (very strong to no off odor).

b>a (differences highly significant).

c>a, b (differences highly significant).

The TBA numbers become progressively lower with longer ooks throughout all storage periods. Reduction in TBA numbers was greatest between 99 and 110°, indicating that the oxidative reaction is affected drastically in this 11° interval. The threshold for sensory detection of rancidity has been found to be a TBA number of approximately 1. The overcooked sample never reached this level in any storage period, and the 110° sample reached it only after 8 days of storage. This is borne out in the sensory scores shown in Table 1.

The percent of pigment remaining after cooking is based on the value for the 80° sample since it has been observed in this laboratory that little or no pigment destruction takes place when meat is heated to temperatures below 90°. The percent of pigment remaining at 0 days of storage indicates that destruction of myoglobin by heat increases progressively as the internal temperature is increased. Only ⅓ of the pigment remains in the overcooked sample. Loss of pigment is greatest in the additional hour of cooking between the 110° and long-cook samples. Pigment loss during the subsequent 8-day storage is related to lipid oxidation (Watts, 1954). In the 3 samples heated to internal temperatures below 100° the loss range is 38–56%, whereas in the 2 heated to above 100° the loss range is 2–15%. Lipid oxidation is proceeding rapidly in the first 3 samples, and only slightly in the last 2.

The sensory scores previously mentioned indicate that the panel members did not detect rancidity to any degree in the 110° and overcooked samples. They were aware of additional odors, however, which they characterized as "strong but not rancid," "sweet," and "like braised meat."

These results seemed to indicate that pigment destruction might be involved in retardation of the oxidative process. However, products such as pork and veal, which have a pigment concentration not very different from the lowest figures obtained here, are capable of rapid oxidation. For this reason, the other alternatives were explored.

Lipid changes in relation to lipid oxidation. To determine whether lipid destruction had taken place during extensive heating, a sample of ground beef round was "overcooked" and another portion of the sample was heated in a boiling water bath to an internal temperature of 70°C. At 0 days of storage the weight of washed lipid was determined. Raw tissue supplied 51 mg of lipid per g of tissue; 70° sample gave 55 mg; and the overcooked sample gave 52 mg. After 3 days of storage the lipids were again extracted and the molar concentration of polyunsaturated fatty acids (PFA) was determined. The 70° sample contained 7.5×10^{-3} moles of PFA per kg tissue, and the overcooked sample contained 8.3×10^{-3} moles per kg. Apparently there was no measurable destruction of PFA by extensive heat treatment.

Antioxidant production in relation to lipid oxidation. Two different types of experiments were used in determining whether an antioxidant was produced during overcooking. The first involved adding 50% and 10% overcooked ground beef round to raw meat of the same sample (50% and 90%, respectively) and heating to 70°C in a boiling water bath along with a 100% raw meat control. The TBA test was performed on these samples initially and after 3 and 6 days of storage. The results are in Table 2, section A.

The overcooked meat appeared to have an antioxidant effect, especially in 50% concentration. After 6 days of storage, the TBA number was ¼ that of the 0% control, whereas ½ would have been expected because of dilution. The depression at 10% concentration might possibly have been due to dilution.

All 4 samples were presented to the panel of judges after 3 days of storage. The statistical evaluations and sensory scores are shown in Table 2. The judges made no comments. These results are in agreement with the TBA numbers.

As a further test of their antioxidant activity, slurries of overcooked meat were used as covering solutions for slices of roasted eye of round. The meat was roasted to an internal temperature of 165°F in a 325°F oven. The roast was cooled rapidly by wrapping in aluminum foil and immersing in ice water, and then sliced thin with a mechanical slicer. The slices were placed in 307×113 cans, and five different covering solutions were added: water control, 50% slurry, 20% slurry, 2% slurry, and a solution of 1% sodium tripolyphosphate plus 0.22% sodium ascorbate. The cans were stored uncovered at refrigerator temperatures. The TBA test was performed after 4 and 8 days of storage. Both controls and the 50% slurry sample were presented to the panel of judges. The data are in Table 2, section B.

The TBA numbers indicate that the sample covered with a 50% slurry was protected from rancidity over the time studied. The judges did not pick up rancid odors in this sample. They made comments such as "roast meat odor," "stronger meaty odor," and "meaty flavor." In comparison with this sample, the polyphosphate-ascorbate control seemed "washed out" to a few judges—the odor was not rancid but was not as intense as that of the 50% slurry sample. The TBA numbers of the 20%

Table 2. TBA numbers and sensory scores.

Experimental variation	Days of storage	TBA number	Sensory score
A) Ground beef samples cooked with over-cooked beef [a]			
100% control	0	0.2	....
	3	0.3	5.6(a)
	6	0.3	....
50%	0	1.0	....
	3	2.6	5.3(b)
	6	3.8	....
10%	0	2.0	....
	3	8.7	3.3(c)
	6	15.	....
0% control	0	1.9	....
	3	10.	3.0(d)
	6	18.	....
B) Roast beef slices stored in slurries of over-cooked beef [b]			
Water control	4	6.7	2.7(a)
	8	11.	2.8
2% slurry	4	5.0	....
	8	9.7	....
20% slurry	4	3.0	....
	8	5.5	....
50% slurry	4	0.7	5.2(b)
	8	1.0	5.5
1% polyphosphate–0.22% ascorbate control	4	0.0	5.6(c)
	8	0.0	5.3
C) Beef slices stored in various covering solutions [c]			
Water control	4	5.9	2.4(a)
	7	12.	....
20% slurry	4	3.5	4.9(b)
	7	5.4	....
20% slurry with 0.5% polyphosphate	4	0.3	5.7(c)
	7	0.2	....
0.5% polyphosphate control	4	0.3	5.7(d)
	7	0.4	....

[a] a, b>c, d (differences significant).

[b] b, c>a (differences highly significant).

[c] b, c, d>a (differences highly significant).

slurry sample are about ½ those of the water control. This concentration could be said to have a moderate antioxidant effect, and the 2% concentration had a very slight effect.

These 2 experiments demonstrate antioxidant formation in overcooked meat. As a by-product of these experiments, slurries of this meat were found to have a pleasant "meaty" odor. An additional experiment was designed to determine the acceptability of roast beef slices stored in a 20% slurry with added antioxidants, and the effectiveness of such a combination against rancidity. The 20% slurry was chosen because it had a mild "meaty" odor and was about the consistency of a gravy. The TBA test was performed on the samples and appropriate controls after 4 and 7 days of storage. The data are in Table 2, section C.

The TBA numbers are very low for the slurry-antioxidant combination and the polyphosphate control, and the antioxidant capacity of the slurry alone is demonstrated again. All four samples were presented to the judges after 4 days of storage. As shown in Table 2, the polyphosphate and combination samples received identical high average sensory scores. The judges commented that the combination sample was "very fresh" and the polyphosphate control "lacked meaty odor." They were asked to rank the samples according to acceptability. Half of the judges ranked the combination sample as the most acceptable, and the other half selected the polyphosphate control. In all cases the water control was least acceptable, and the 20% slurry next.

DISCUSSION

Uncured canned meats can be stabilized against oxidative rancidity, without the addition of antioxidants, by heat treatment in excess of heat sterilization. This effect is due to the production of antioxidants in the meat itself at high temperatures. No attempt was made to ascertain the nature of the antioxidant or its location within the meat. Perhaps sulfur compounds, resulting from protein breakdown at high temperatures, retard the oxidative process; or the various constituents of the meat may be interacting in some unknown way to produce antioxidants. Triebold (1945) noted that the various formula components of cereal products have a definite effect on the stability of the product. Although the fatty acid composition of the shortening is important, the product stability cannot be predicted from the fat stability. If the antioxidant-active material is located in the soluble fraction of the meat, it should be possible to concentrate and characterize.

For aesthetic and nutritional reasons, commercial use of extensive heat treatment for stabilizing uncured canned meats cannot be encouraged. Thiamine would be almost completely destroyed, and the appearance and odor of the meat would be changed, resulting in a "caramelized" color and strong "canned meat" odor. Actually, some overprocessing occurs in commercial practice, because the National Canners Association does not publish minimum process times for meats, as it does for fruits and vegetables (Frazier, 1958). Good margins of safety are usually allowed. Canned cured meat products have several advantages, undoubtedly accounting for their prevalence on the market. The curing ingredients are known to aid in the destruction of spores of anaerobic bacteria by heat and to inhibit the germination of surviving spores (Frazier, 1958). Less processing time is therefore required, and less thiamine destruction takes place. In addition, rancidity is controlled because of conversion of the heme pigments to the catalytically inactive cured-meat pigment (Younathan and Watts, 1959).

The information provided by these experiments, in addition to giving insight into the oxidative reaction at high temperatures, may have some application. Dilute slurries of overcooked meat provide a pleasant "meaty" odor that enhances the aroma of sliced meat. If the additional needed protection against oxidation of the meat slices can be obtained from antioxidants, either chemical or natural, the use of such slurries as a part of the gravy for prepared dinners might improve the acceptability of such products. It would be of interest to try vegetable extracts, such as those from the skins of onions (Lewis and Watts, 1958), known to have antioxidant capacity, in combination with overprocessed meat.

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